Vol. 68, pp. 125-129, 1986

Allogeneic Transplantation of Normal Epidermal Cells and Squamous Cell Carcinomas in SENCAR Mice

by Claudio J. Conti,* JoAnn W. Fries,* Claudio M. Aldaz,* Andres J. P. Klein-Szanto,*† and Thomas J. Slaga*

A method used in our laboratory for the transplantation of single cell suspensions of epidermis and skin carcinomas is described. Silicone chambers were placed into a subcutaneous granulation tissue formed by the implantation of a glass disk. The skin was closed on top of the chamber by using Michael autoclips. Cells were injected 24 to 48 hr later through the skin using a syringe and needle. The neoformation of epithelia with adnexa was observed when newborn epidermal cells were injected. When a fresh cell suspension of squamous cell carcinoma was used, a typical differentiated carcinoma was formed within 2 to 4 weeks. However, after 4 weeks, some of the grafts showed mild signs of rejection. The technique described is a useful system to transplant squamous cell carcinomas and can also be used as a rapid assay for malignancy.

Introduction

Studies from several laboratories have shown that isolated epidermal cells, either fresh or cultured, may be reimplanted into appropriate host animals. Yuspa et al. (*1*) developed a silicone chamber that produces a protected area in the panniculus carnosum. Embryonal keratinocyte cultures, transferred into these chambers, form a hyperplastic epidermis with normal appendages and hair follicles. A modification of this method was reported by Worst et al. (*2*). They implanted a smaller silicone chamber into a highly vascularized granulation tissue formed by the implantation of a glass disk into the subcutaneous tissue. These authors were able to obtain an organotypic reorganization of normal and malignant mouse epidermal cultures after transplantation to syngeneic or allogeneic recipients (*2–4*).

A similar system was used by Mackenzie to protect heterotypic transplants from skin and oral mucosa (*5*). The pieces of tissue were placed in a granulation tissue formed with the glass disk technique, and a small polyethlene chamber was used to cover and protect the implant. In this system, the skin of the host was closed on top of the chamber, thus completely covering the implant. In this way the graft was protected from infection and dessication, which are two of the problems with the previously described systems.

Tumor transplantation is an important tool in tumor biology. It permits the study of a tumor beyond the lifespan of the host and allows the tumorous mass to be expanded for different analyses. However, transplantation of chemically induced mouse skin tumors does not seem to be easily accomplished. Early reports have shown that a few coal-tar-induced (*6*), benzo(a)pyrene-induced (*7*), or 3-methylcholanthrene-induced (*8*), tumors can be transplanted into syngeneic mice. Conversely, 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin carcinomas cannot be transplanted (*9*). In addition, no successful transplants of skin tumors produced by two-stage carcinogenesis into outbred mouse stocks such as SENCAR have been reported. In this paper we present a modification of the above-described techniques that allows a successful transplantation of fresh and cultured epithelial cells and freshly isolated cells from squamous cell carcinoma in SENCAR mice.

Materials and Methods

Preparation of Tumor Single-Cell Suspensions

Papillomas and carcinomas were induced on the dorsal skin of SENCAR mice (Harlan, Sprague-Dawley) by using a two-stage chemical carcinogenesis protocol with 10 nmole of DMBA as the initiator, followed twice weekly by a promoter treatment with 2 μg of 12-O-tetradecanoylphorbol-13-acetate (TPA) in 200 μL of acetone. After 10 to 20 weeks the first papillomas arise,

*The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX 78957.

†Present address: Fox Chase Cancer Center, Philadelphia, PA 19111.

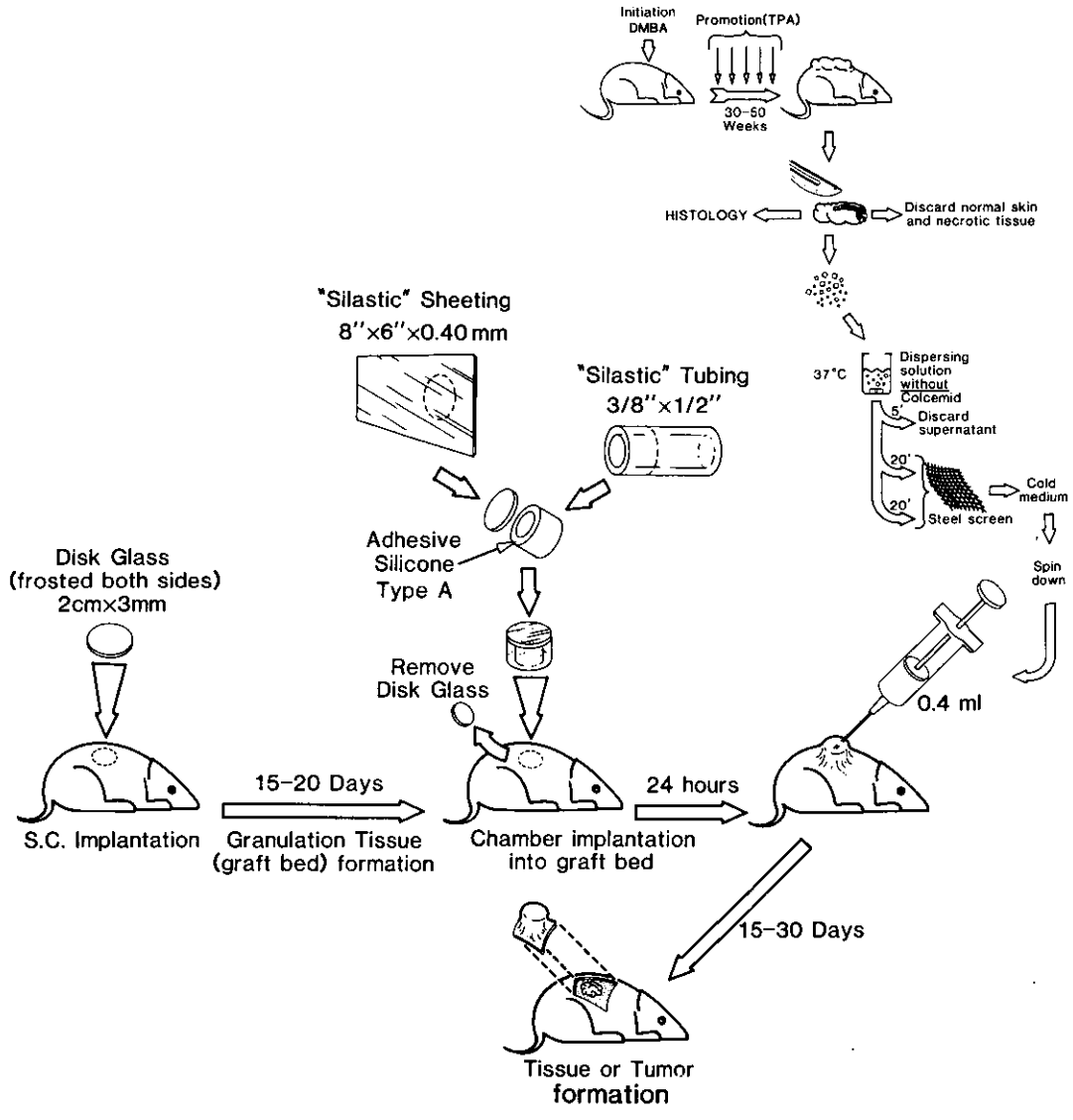

FIGURE 1. Schematic representation of the technique used for the transplantation of skin tumors in SENCAR mice.

and at 20 to 60 weeks, carcinomas develop in 45% of the mice (*10*). The tumors selected for transplantation were dissected and enzymatically dispersed using a technique developed in our laboratory for obtaining chromosome preparations (*11*) and short-term cultures from skin carcinoma (unpublished results). The tissues were immediately washed in Dulbecco Modified Eagle (DME) medium with Hepes buffer, and the necrotic tissue fragments separated. The sample was later scraped with a scapel, thus producing a dissociated preparation. The mechanically dispersed tissue was treated with a dispersing solution containing 0.25% trypsin (Sigma Co.), 0.25% type-II collagenase (Sigma Co.), 0.1% hyaluronidase (Sigma Co.), and 0.5% bovine albumin in a modified Hanks' solution with Hepes buffer (pH 7.4) prewarmed at 37°C. The tissue was stirred slowly at the same temperature with a magnetic bar. The first 10-min wash, enriched in squamous, necrotic and inflammatory cells was discarded. Two more washes of 20 to 25 min were processed separately immediately after being obtained. The collected supernatant was filtered through a steel screen mesh. The enzymatic treatment was stopped using a cold DME medium with 10% fetal calf serum. The resulting material was centrifuged at 1000 rpm, and the pellets were resuspended in fresh medium.

Preparation of Newborn Single-Cell Suspension

Single-cell suspensions of newborn epidermis were obtained by the method described by Yuspa (*1*). Briefly, the skins were floated in 0.25% trypsin overnight at 4°C. Dermis was separated from the epidermis using sterile forceps and tweezers. The epidermis was washed in cold tissue culture medium with 10% fetal calf serum,

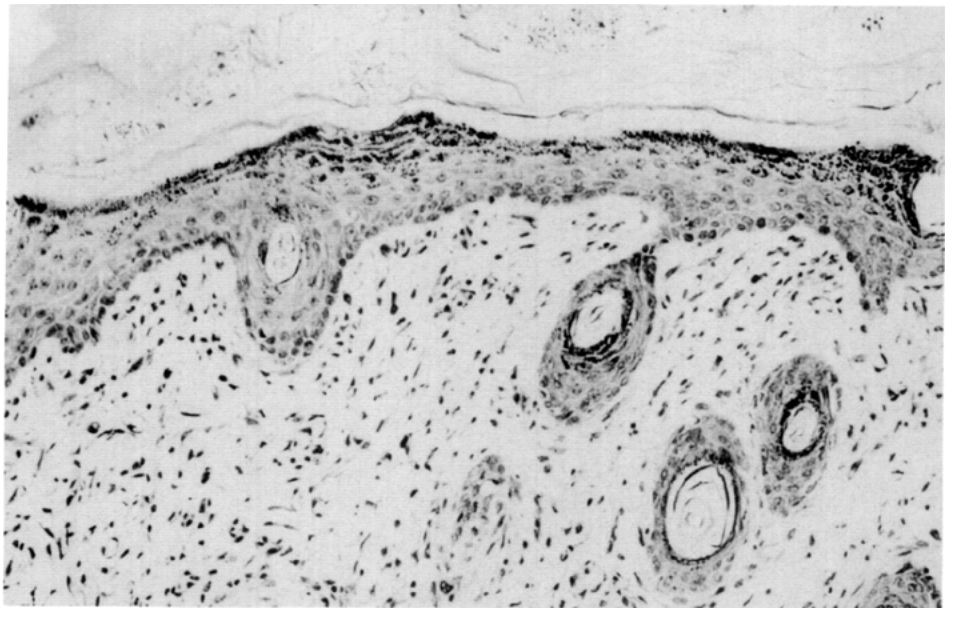

FIGURE 2. 3-week old transplant of SENCAR mouse skin implanted in 6-week-old SENCAR mouse. H & E, ×70.

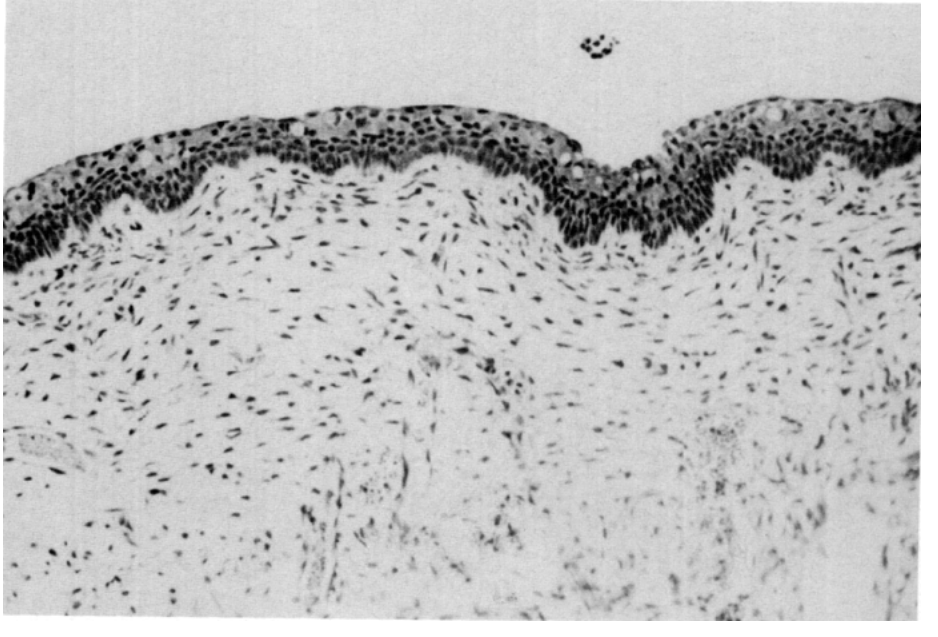

FIGURE 3. 3-week old transplant of SENCAR mouse vagina in a chamber. H & E, ×70.

minced with scissors and stirred in the medium for 30 min. The cell suspension was filtered through a nylon mesh, centrifuged, and resuspended in fresh medium without serum.

Chamber Preparation

Silicone chambers were manufactured in our laboratory as described in Figure 1. A ⅜ × ½ in. tube (Dow Corning) was cut in 1-cm pieces, and one end was closed with circular silicone pieces obtained by cutting 8 in. × 6 in. × 0.4 mm medical-grade silicione sheeting (Dow Corning). Both silicone pieces were glued together with Type A silicone medical adhesive (Dow Corning).

Cells and Tissue Implantation

A suitable graft bedding was prepared by implanting a glass disk subcutaneously as described by Worst et al. and Fusenig et al. (*2,3*). Between 2 and 3 weeks after

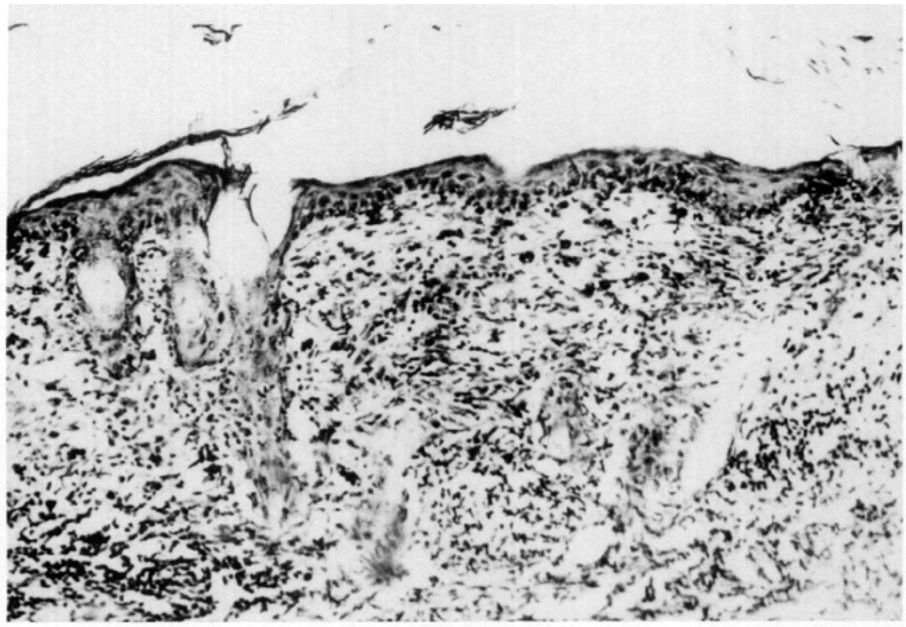

FIGURE 4. Reconstituted epithelium in chambers injected with 10^6 cells from SENCAR newborn epidermis. H & E, × 70.

implantation, an incision was made over the glass disk and the disk was removed from the animals. The chamber was placed inside the pocket of granulation tissue that had formed around the glass and the skin was then sutured over the chamber with clamps. After 24 to 48 hr, cell suspensions were injected through the skin and into the chamber using a syringe and a 25-G needle. Approximately 1 to 2 × 10^6 cells, resuspended in 0.4-mL medium without serum, were injected into each chamber.

Explants of tissues were grafted in the chambers directly on the granulation tissue immediately after removal of the glass disk (*5*). The explants were covered by the chambers, and the skin was closed over the chambers as described previously.

After 2 to 6 weeks the animals were sacrificed; the chambers were dissected out from the tissue bed; and the tissues were fixed in buffered neutral formalin, embedded in paraffin, and stained with hematoxylin and eosin.

Results and Discussion

The method described above to implant normal and tumor cells into SENCAR mice is essentially similar to the technique described by Mackenzie (*5*). However, we have developed a chamber that can be easily prepared in the laboratory and seems more suitable for cell growth. The original polyethylene chambers described by Mackenzie were excellent for supporting the growth of full tissue explants but in our hands were not adequate for the growth and tissue reorganization of single-cell suspensions.

Explants of skin and vagina were grown in the chambers for a period of 4 weeks. Even when some of the explants exhibited signs of immunological rejection, most of the explants looked healthy after 3 weeks (Figs. 2 and 3). Most of the explants showed a large outgrowth of the epithelial cells covering the granulation tissue.

Isolated epithelial cells from newborn mice injected into the chambers formed an almost normal epithelium within 2 weeks (Fig. 4). Rudimentary hair follicles and glands were observed in all the cases. The presence of skin adnexa, previously described by Yuspa (*1*), has been ascribed to the inductive properties of dermal fibroblasts (*4*). In our case, the presence of skin adnexa suggests fibroblast contamination of our epidermal preparations. The possibility that the newly formed skin can be produced by an outgrowth of the host skin can be ruled out because reparative epidermal growth is not usually accompanied by adnexa, because there is no continuity between the skin and the newly formed epithelium, and because chambers that have not been inoculated with epithelial cells do not produce epithelium. We also ruled out the possibility that the epithelium came from remnants of hair follicles because, as previously stated, empty chambers do not produce epithelium and because we have never histologically observed hair follicles trapped in the granulation tissue.

Cells from skin squamous cell carcinomas were isolated by a method developed in our laboratory and used successfully to obtain tumor karyotypes (*11*). This method has also been used to obtain viable cells for short-term culture (Conti et al., unpublished results). The injection of this cell suspension produced the growth of neoplastic tissues within 2 weeks (Fig. 5). The tumors were well-differentiated squamous cell carcinomas resembling the original neoplasia. When tu-

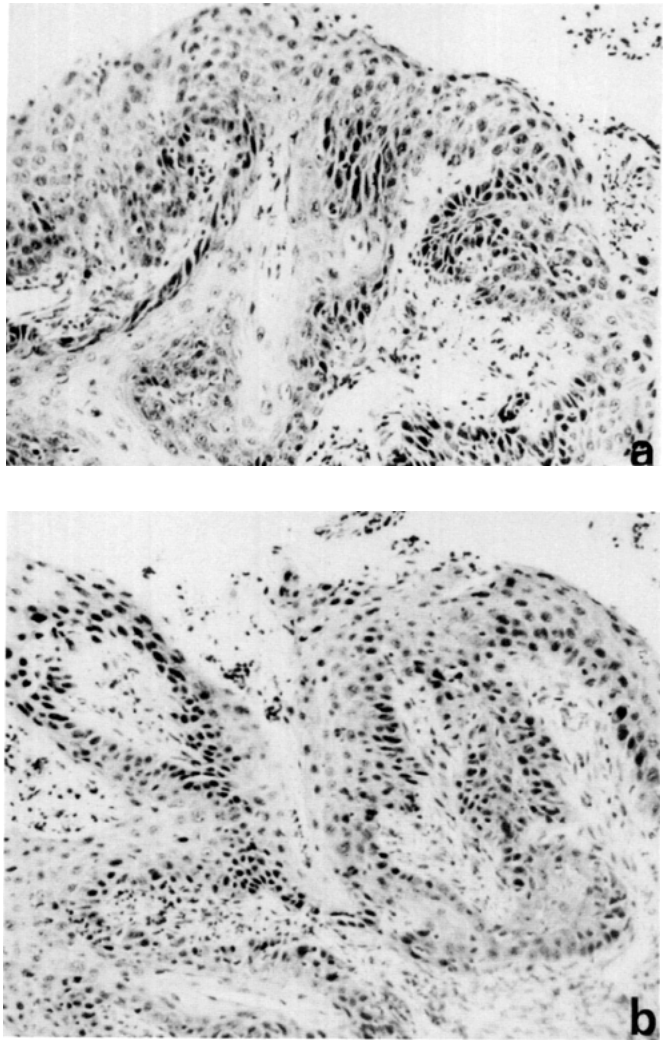

FIGURE 5. (*a*) Squamous cell carcinoma induced in SENCAR mice by a two-stage carcinogenesis protocol; (*b*) the same carcinoma after being dissociated and grown in the chamber for 2 weeks. H & E, ×100.

mors were allowed to grow over the fifth week they started showing signs of rejection.

The possibility of implanting epithelial cells in allogeneic hosts was previously explored by Worst et al. and Fusenig et al. (*2,3*). They found that pure epithelial cells are not rejected when implanted in chambers up to at least 7 weeks. Similarly, we have been able to grow normal and tumor cells from the outbred SENCAR stock for a minimum of 5 weeks. Some of our preliminary experiments indicate that beyond the fifth week, rejection of the graft, especially tumor cells, may take place. However, since one of the great advantages of the chamber assay is the fast cell growth, a 5-week period seems to be adequate for most tumor transplantation studies. The chamber system also appears very promising as a fast assay for malignant transformation of cell cultures. Preliminary studies from our laboratory have shown that keratinocytes transformed *in vitro* form an epithelial structure with areas showing invasion within 2 weeks.

The authors wish to thank Ms. Joyce Mayhugh, Mr. John Riley, and Ms. Judy Ing for the preparation of this manuscript. We also want to acknowledge the contributions of Dr. S. Yuspa and Dr. I. Mackenzie in technical and scientific advice and samples of their chambers. This paper was supported by NIH Grant 5511 IN 42, NIH CA 38863, and NIH CA 34890. The work decribed in this paper was not funded by EPA and no official endorsement should be inferred.

REFERENCES

1. Yuspa, S. H., Morgan, D. L., Walker, R. J., and Bates, R. B. The growth of fetal mouse skin in cell culture and transplantation of F1 mice. J. Invest. Dermatol. 55: 379–389 (1970).
2. Worst, P. K. M., Valentine, E. A., and Fusenig, N. E. Formation of epidermis after reimplantation of pure primary epidermal cell cultures from perinatal mouse skin. J. Natl. Cancer Inst. 53: 1061–1064 (1974).
3. Fusenig, N. E., Valentine, E. A., and Worst, P. K. M. Growth behaviour of normal and transformed mouse epidermal cells after *reimplantation in vivo*. In: Tissue Culture in Medical Research, Number II (R. J. Richards and K. T. Rajan, Eds.), Pergamon Press, Oxford, 1980, pp. 87–95.
4. Worst, P. K. M., Mackenzie, I. C., and Fusenig, N. E. Reformation of organized epidermal structure by transplantation of suspensions and cultures of epidermal and dermal cells. Cell Tissue Res. 225: 65–77 (1982).
5. Mackenzie, I. C., and Hill, M. W. Maintenance of regionally specific patterns of cell proliferation and differentiation in transplanted skin and oral mucosa. Cell Tissue Res. 219: 597–607 (1981).
6. Mottram, J. C. On the correlation between malignancy and the rate of growth of tar warts in mice. Am. J. Cancer 22: 801–830 (1934).
7. Salaman, M. H. Transplantable benzpyrene induced skin carcinomata of mice. J. Pathol. 55: 381–382 (1943).
8. Cooper, Z. K., Firminger, H. I., and Reller, H. C. Transplantable methylcholanthrene skin carcinomas of mice. Cancer Res. 4: 617–621 (1944).
9. Räsä, O. Transplantability of chemically induced skin tumors in syngeneic strain of mice, rats and guinea pigs. Exptl. Pathol. 17: 121–127 (1979).
10. Slaga, T. J., Fischer, S. M., Weeks, C. E., and Klein-Szanto, A. J. P. Cellular and biochemical mechanisms of mouse skin tumor promoters. Rev. Biochem. Toxicol. 3: 231–281 (1981).
11. Aldaz, C. M., Conti, C. J., Klein-Szanto, A. J. P. and Slaga, T. J. A direct cytogenetic technique for mouse skin carcinomas and papillomas. Cancer Genet. Cytogen. 20: 223–229 (1986).